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MODIFICATION OF WHEAT AND OTHER PLANT-BASED BINDER SYSTEMS FOR USE IN FRESH MEAT PRODUCTS

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ADF Project 99000072
Final Report

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ABSTRACT/SUMMARY

The objective of this project was to investigate the effect of soy- and wheat-based binders on the colour degradation of fresh ground beef. The *in vitro* and ground beef study showed that binders affect the rate of red colour degradation. Inclusion of commercial wheat gluten and wheat flour, soy protein concentrate, soy protein-isolate and deheated mustard flour at a 4.5% (w/w) level had an accelerated effect on red colour degradation of ground beef. Micronized wheat flour and toasted wheat crumb, which are subjected to a heat treatment during the preparation resulted in the best retention of fresh colour of ground beef. Soy protein-concentrate and -isolate as well as deheated mustard flour effectively suppressed the oxidation of unsaturated lipids and thus the development of off- flavours in meat.

EXECUTIVE SUMMARY

Consumers use colour as an indicator of the freshness of meat products: A bright cherry red colour is preferred over brown when they make purchase decisions of fresh red meat cuts and products. Oxymyoglobin (oxyMb), the pigment molecule responsible for the bright red colour in meat, is quite unstable. It can deteriorate rapidly and turn to brown metmyoglobin (metMb) during storage. In Canada ground meat is the most popular form of beef, and is available in bulk or as products such as burgers, patties and sausages. At the retail level, storage temperature, illumination conditions, packaging material and atmospheric composition are carefully manipulated in order to delay brown colour formation of these products. Products made from comminuted meat contain seasonings that include spices, salt and fillers/binders. Binders are usually plant-based materials and are incorporated for enhanced product functionality and reduced formulation costs. Soy protein products and toasted wheat crumb are the most popular fillers used by the meat processors. Wheat flour, an inexpensive binder, has been implicated to cause rapid colour loss in fresh meat products. This study was undertaken to benefit meat processors by increasing our understanding of how binders influence meat colour. We also examined alternative filler/binder ingredients to use in fresh meat products that can result prolonged red colour stability. In particular, our study was aimed at finding suitable economical wheat-based binders with good functionalities and with the hope that these could reduce formulation costs compared to those involved when using soy protein ingredients.

This study was conducted using two separate systems: The first involved using purified myoglobin, which is responsible for the colour of meat, while the second used a burger-type, fresh, ground beef product. The fillers/binders used for the two systems were soy (soy protein concentrate and isolate both commercial grade), wheat (commercial grade-wheat flour, -wheat gluten and -toasted wheat crumb, wheat flour from micronized whole wheat, whole wheat flour and laboratory milled wheat flour) and commercial-grade deheated mustard flour (*i.e.,* heat treated to reduce pungency). All these binders were added at 4.5% (w/w) level on the basis of total weight.

For the myoglobin system, the bright cherry red colour of oxyMb was prepared by chemically reducing metMb in the laboratory. The effect of the binders on the conversion of the red pigment to brown was monitored with or without unsaturated lipids in the system. Presence of toasted wheat crumb and micronized wheat flour was able to slow down the brown colour

formation when lipids were not added to the system. This study indicated that the binders have a direct effect on the oxidation rate of oxyMb. When unsaturated phospholipids were present in the system all binders showed an accelerated effect on brown colour formation. Among the binders tested wheat gluten and soy protein ingredients resulted in the fastest rate of red colour reduction in the liposomes.

We also studied colour change via inter-conversion of myoglobin and lipid oxidation in burger-type products. This work showed what actually happens when these binders are incorporated into ground beef. By tracking the contents of oxyMb and metMb in meat it was found that among the wheat flour ingredients, toasted wheat crumb and micronized wheat flour can slow down the formation of metMb and delay brown coloration. Soy protein ingredients and deheated mustard flour had an accelerated effect on oxyMb depletion. Instrumental evaluation of product colour that simulates human eye perception, indicated that redness of the product stayed longer when toasted wheat crumb, micronized wheat flour and whole wheat flour were incorporated in the product rather than with soy protein products or deheated mustard flour.

It is an established fact that oxidation of both unsaturated lipids and oxyMb are interrelated. When the antioxidant/reductant ascorbic acid was incorporated at 300 ppm level to the formulation, beef burgers had extended colour stability and a very low concentration of lipid oxidation products was formed. However when the binders were present, these two oxidation processes did not occur in parallel; accelerated lipid oxidation was not always accompanied by fast depletion of oxyMb (*i.e.,* pigment oxidation). Soy protein concentrate and isolate and deheated mustard flour were very effective in suppressing the formulation of lipid oxidation products but not the oxidation of meat pigments. On the other hand toasted wheat crumb and micronized whole-wheat flour were less effective antioxidants than soy ingredients or deheated mustard flour but were able to slow down red colour fading of fresh ground beef.

The antioxidant activity of soy ingredients and deheated mustard flour, which was exhibited in fresh meat, matched well with their antioxidative potential as determined by antioxidant indicators. All wheat flour ingredients had a moderate antioxidative potential with the exception of toasted wheat crumb and wheat gluten. Except for micronized wheat flour and toasted wheat crumb, other binders showed moderate oxidative enzyme activity, while soy protein ingredients and deheated mustard flour exhibited negligible activity. The positive effect of toasted wheat crumb and micronized whole wheat flour on fresh meat colour stability may come from the heat treatment to which they are subjected during ingredient processing. It is an

indication that heat treatment may enhance the ability of wheat flour-based ingredients to stabilize colour of fresh ground meat containing products. In toasted wheat crumb, the presence of bicarbonate salts may also increase the pH of meat slightly and will help to maintain a favourable environment to slow down the autooxidation of oxyMb. With the other binders such as micronized wheat flour, however, it is difficult to point out one factor that can slow down metMb formation. Soy ingredients and deheated mustard flour are rich in natural antioxidant phenolic compounds, which may under go oxidation/reduction reactions and thereby impart antioxidative activity towards unsaturated lipids. Possibly the intermediate products of these reactions may act as prooxidants or catalysts toward oxyMb oxidation. The following points can be summarized from this study.

- ★ At 4.5% level (by weight) soy protein concentrate and isolate accelerated the red colour fading of products made of fresh ground beef containing 1% salt and 5.5% water.
- ★ At the same level, toasted wheat crumb and micronized wheat flour delayed red colour fading thus prolonging fresh meat colour. Deheated mustard flour at 4.5% (w/w) level of addition accelerated red colour degradation, and therefore, care should be taken when deheated mustard flour is included in fresh meat product formulations particularly, if colour is a concern. This information will be communicated to the industry in several forms (*e.g.*, presentations, fact sheets).
- ★ In contrast to the accelerated red colour degradation, soy protein products and deheated mustard flour are very good agents for suppression of off-flavour formation of fresh ground beef products. These ingredients are useful in products where fresh meat colour is not a concern but prevention of flavour defects is (*e.g.*, long term frozen storage). A study is needed to determine the level of incorporation that can balance both colour and oxidation stability.
- ★ Micronized wheat flour should be further studied to establish the optimum level of addition to improve colour stability as well as other sensory aspects such as taste and texture.

BACKGROUND:

Colour is the visually encountered quality parameter of fresh meat. In red meat the bright, cherry red colour is normally used as an indicator of freshness by the consumer. Therefore, the colour directly influences the purchasing decision of fresh meat and is a critical issue at the retail level. The bright cherry red colour of fresh meat eventually fades to an unacceptable brown colour that is hard to control. Several practices ranging from animal management, handling during slaughter, packaging and storage after conversion of muscle to meat are integrated carefully in order to delay the conversion of red colour of fresh meat to brown. At the retail level fresh meat is available as cuts (*e.g.*, chunks, steaks, slices), ground or minced and the products thereof (*e.g.*, burgers, patties or sausages). During the preparation process, the extent of oxygenation increases, the meat begins to bloom and appears bright red, however, red colour degradation is also enhanced. In Canada, ground beef accounts for about 50% of all kg volume and 30% of all dollar volumes sold at the retail level (BIC, 2003) and is the most popular form of beef consumed. During formation of products from comminuted meats a variety of ingredients such as salt and seasonings are added, and unintentionally atmospheric oxygen. The following sections briefly discuss the chemistry of meat pigments, their conversion and possible effects of product formulation on fresh colour.

Chemical basis of meat colour

Myoglobin (Mb) is the heme protein primarily found in meat. Hemoglobin and cytochromes are also heme proteins found in muscle tissues but their contribution to fresh meat colour is minimal as compared to Mb. Beef contains a higher percentage of Mb than poultry tissues thus is redder in colour. Myoglobin is a globular protein that is made up of a single polypeptide chain (*i.e.*, globin consists of 153 amino acid residues and a prosthetic heme group,

an iron(II) protophorphyrin-IX complex). The heme molecule is an organometalic compound: The central iron atom coordinates with the nitrogen atoms of four pyrrole rings and one coordination site with the imidazole nitrogen of the proximal histidine residue of the globin protein. The other coordination site is available to bind an oxygen molecule, a hydroxyl group or a water molecule. The iron atom in the middle of the heme group plays a crucial role in determining the colour of fresh meat. It may exist in a reduced ferrous (+2) or oxidized ferric (+3) form, thus changing the chemical state of Mb and eventually dictating the light absorbing properties. When iron is in Fe(+2) state with a free sixth coordination site (no ligand is bound), Mb is in the deoxymyoglobin (deoxyMb) form. Meat appears purplish-red in colour when deoxyMb is the predominant pigment form (*e.g.*, vacuum packed fresh beef). When a molecule of oxygen occupies the sixth binding site of the ferrous heme, oxymyoglobin (oxyMb) results and it is responsible for the desirable bright, cherry-red appearance of fresh meat. The deoxy and oxy forms of Mb readily oxidize to the undesirable brownish-red pigment of metmyoglobin (metMb); in which the iron atom is converted to the ferric state and water (or a hydroxyl group) occupies the sixth coordination site. Metmyoglobin is incapable of binding oxygen. If metMb predominates, meat becomes brown and loses its saleable value. Figure 1 illustrates this inter-conversion of Mb in fresh meat.

Why does fresh meat colour change?

In living tissues of the animal Mb serves both oxygen-storage and -delivery functions. The biochemical environment within the tissue controls oxygen supply, oxidation-reduction reactions and other favourable conditions necessary to maintain Mb predominantly in deoxy- and oxy-form. In post-mortem muscle this regulatory mechanism is not operating any longer and the balance between prooxidative and antioxidative factors favours oxidation of the Mb

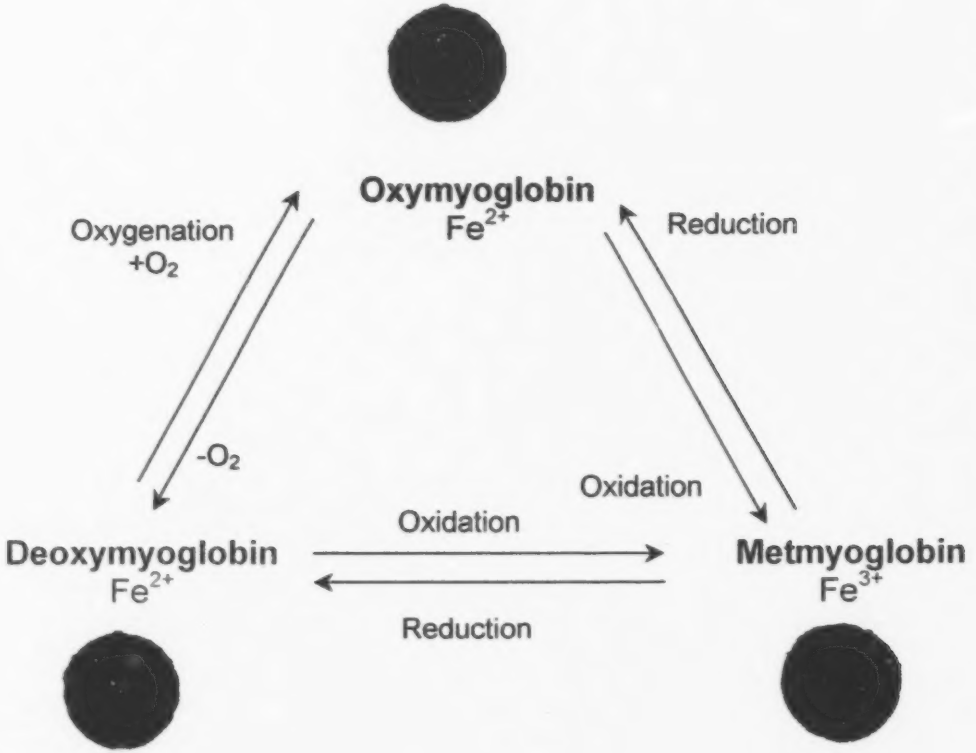

Figure 1. Interconversion of myoglobin of meat. Pictures indicate the colour of meat when the respective molecular form predominates.

molecule. The kinetics of this conversion process is governed by genetic factors such as species, type of muscle, physiological condition of the animal (*i.e.*, age, dietary regime, sex, stress) and post mortem handling conditions (*e.g.*, temperature, relative humidity, packaging, illumination). Today, many factors can be manipulated to prolong the colour stability of fresh meat by slowing down the chemical conversion of deoxyMb and oxyMb to metMb.

In ground or minced meat this situation is different than in meat cuts. Chopping, grinding and mixing at least partially disrupt organization at the cellular level and incorporate atmospheric oxygen into the meat. Due to contact with atmospheric oxygen, a fraction of the deoxyMb is oxygenated and converted to oxyMb, thus giving the bright red cherry colour or "bloom" to the meat. Brighter muscle colour results because most of the heme pigment exists as either deoxyMb or oxyMb. The conversion of deoxyMb and oxyMb to metMb depends essentially on the rate of deoxyMb/oxyMb autooxidation, the oxygen consumption rate (in combination with ultimate pH), lipid oxidation, and enzymic/nonenzymic metMb reduction capacity.

Exposed to oxygen, Mb is slowly and continuously oxidized to metMb in fresh meat. The autoxidation rate of oxyMb depends on oxygen partial pressure, pH of meat, temperature of storage, oxidation/reduction mediators and presence of salts, and catalytic amounts of heavy metals (Renerre, 2000). A lower ultimate pH of meat is detrimental to oxygenation and increases the rate of autooxidation while neutral pH is preferred for colour maintenance, but increases microbial growth. Additionally, globin denatures at pH values below 5.0. Enzymic reduction of metMb is far less effective at low pH values. In general, meat of low ultimate pH discolours faster than that of high ultimate pH. In addition, the change in the redox potential that occurs in post-mortem muscle influences the chemical environment of the tissues.

The oxygen consumption and diffusion rate and partial pressure of oxygen at the meat

surface determine the formation of oxyMb and also the penetration depth (or extent) oxygenation of Mb. When ground meat is wrapped in an oxygen permeable membrane and stored aerobically, the surface layer of meat blooms. At a few mm below the surface, there is a region where the partial pressure is in the optimum range for the formation of a metMb layer. With time, oxyMb oxidizes to metMb and becomes visible at the surface and the meat becomes brown.

The enzyme dependant reducing capacity of muscle is also an important factor in determining how fast metMb can be reduced to deoxyMb, even if it happens slowly. Some muscles discolour at faster rates than others. In muscles with high metMb-reducing activity, higher quantities of reducing equivalents (*e.g.*, reduced nicotinamide adenine dinucleotide; NADH) are present; thus, the iron of Mb remains in the reduced state longer. As meat ages the metMb reducing ability diminishes.

Oxidation of unsaturated lipids of tissue membranes and meat pigments are closely coupled. Myoglobin is a sarcoplasmic protein and is located in close proximity to membranes of sub-cellular organelles. Any physical or oxidative damage that happens to the cytosolic and membrane components may directly influence the oxidation status of Mb and lipids. Cellular and sub-cellular membrane lipids are particularly susceptible to oxidation due to the high concentration of polyunsaturated fatty acids, close proximity to oxygen, transition metal ions and peroxidase enzymes. Red muscles are generally considered to contain more lipids than white muscles, but these lipids tend to be more unsaturated in nature. Some researchers contend that lipid oxidation in muscle tissue is attributed to heme compounds or free iron. However, a number of studies have shown that lipid oxidation is a promoter of oxidation of oxyMb (Lin and Hultin 1977; Faustman and Cassens, 1990; Renerre, 2000). Consequently, the colour stability of fresh meat can be enhanced by the addition of chain breaking antioxidants such as vitamin E or

ascorbate (Yin et al. 1993). In muscle tissues, natural antioxidative compounds such as alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C) are located close to membrane bound enzymes (*e.g.*, NADPH oxidase) that generate free radicals, and they can act to protect membrane lipids by scavenging free radicals. The radical species produced during membrane phospholipid (PL) oxidation may act to promote pigment oxidation. When endogenous vitamin E and C can no longer participate in the oxidation-reduction system, the oxidation of unsaturated lipids continues and more alkoxy and peroxy radicals are generated. These peroxy radicals are prooxidants in the reaction of oxyMb conversion to metMb.

All of the above factors create an environment that enhances oxyMb oxidation reactions in addition to the autoxidation. Therefore, any treatment or additive that is incorporated in subsequent product formulations has a strong influence on the rate of oxidation and subsequently the red colour change (brown colour formation) of fresh meat. Signs of brownish discoloration occur when at least 30-40% of the deoxyMb and/or oxyMb pigments in a given area of the muscle surface become oxidized to metMb. If the meat colour is dark red, purple, or worse yet brown, the beef cut is not considered acceptable in freshness and usually will not be used (cooked/served) at the food service level or purchased at the retail meat market at full price (Smith *et al.*, 2000). Therefore, preventing brown metMb accumulation by delaying pigment oxidation, and/or enhancing reduction of oxidized pigment represents a reasonable approach for preserving the saleable quality of fresh processed meat products.

Use of binders and extenders in fresh meat products

At the retail level ground meat is available as bulk or fresh products made of comminuted meat. According to the product identity specified by the Canadian Meat Inspection Act, fresh, boneless and comminuted meat containing products such as burgers, patties, meatballs and

breakfast and dinner sausages may contain fillers/binders or seasonings. A seasoning is considered as a filler when it contributes more than 1% protein to the finished product (Canadian Meat Inspection Act, 1990). Product designers can manipulate this point to include fillers/binders for various reasons rather than to serve as seasonings. In these products the cook yield is an important consideration for both the consumer and producer. Customers like to see the product remain in same size and shape after it's cooked, or at least to have less purge. In terms of ingredient functionality: the ability of the filler/binder to bind water and lipids that is expressed during thermal denaturation of muscle protein helps to prevent liquid loss. Therefore, the cooking yield is improved by reducing the amount of liquid lost during thermal processing; cohesiveness and shape of the cooked product is also maintained. Wheat flour, soy protein products (concentrate and isolate) perform very well in such applications because of the water holding and texture modifying properties of their proteins and/or polysaccharides. Another advantage is that these plant-based ingredients can be incorporated to increase protein content (*i.e.*, to reduce the lean meat fraction of the product), improve the nutritional and caloric value and also reduce formulation cost of the product. At present toasted wheat crumb (*i.e.*, heat-treated wheat flour with sodium bicarbonate), soy protein ingredients and modified wheat flour (*i.e.*, protein content enriched up to 40% by mixing wheat gluten) are the predominantly employed binders in fresh meat product formulations. These ingredients have their own drawbacks. For example, toasted wheat crumb gives a lower cook yield than soy proteins or modified wheat flour, and soy proteins offer excellent functionality but significantly increase the dollar value of the product compared to wheat ingredients. Modified wheat flour imparts comparable functionality to that of soy proteins but at a low cost. Unfortunately, the products containing modified wheat flour have very low fresh meat colour stability.

As discussed earlier, comminuted meats tend to turn brown and go rancid more rapidly than whole muscle cuts. When products such as burgers and patties are formed, ground meat is handled aerobically. Mixing operations during preparation incorporate atmospheric oxygen into the product and help to oxygenate deoxyMb, resulting in a cherry red colour; however, maintaining this colour is not easy. In addition, the aerobic handling and oxidative compounds in the added ingredients may accelerate this process. Therefore maintaining the red colour of fresh meat products to which binders have been added is a challenge. This study was carried out to investigate how wheat and alternative plant-based binders/extenders could be modified to prolong fresh meat colour without losing their excellent functionalities.

EXPERIMENTAL

Experiments were performed using two systems. An *in-vitro* model study was conducted to understand the effect on oxyMb in a controlled environment. The study of ground beef product was designed to understand the fate of Mb species in the actual meat environment.

Materials

Binders:

- Wheat flour (commercial) (WF), wheat gluten (WG), toasted wheat crumb (TWC), soy protein concentrate (SPC), soy protein isolate (SPI) and deheated mustard flour (DMF) were acquired from UFL Foods Inc., Toronto, ON.
- Whole wheat flour (WWF) and micronized (IR treated) wheat flour (MWWF) were obtained from InfraReady Foods, Saskatoon, SK.
- Wheat flour (70% extraction, Laboratory milled, Ac Elsa) (UWF) was from the Crop Development Centre, University of Saskatchewan, Saskatoon, SK.

Meat:

Beef inside round (one inch trimmed for fat), Canada A grade (slaughtered by XL Meats Calgary, AB) was obtained from Mitchell's Gourmet Foods Inc., Saskatoon, SK. The meat was stored at –30°C and thawed at –1°C for 48 h before use.

Myoglobin:

Horse skeletal muscle myogobin, 98% pure, was purchased from Sigma–Aldrich Canada Ltd., Oakville, ON. MetMb was chemically reduced with sodium dithionite while oxygenating, and then excess dithionite was removed with a mixed ion exchange resin. OxyMb was freshly prepared and the concentration was calculated daily.

Phospholipids:

- 1,2-Dioleoyl-sn-glycero-3-phosphocholine was acquired from Sigma–Aldrich Canada Ltd., Oakville, ON.
- 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine and 1,2-Dilinolenoyl-sn-glycero-3-phosphocholine were purchased from Avanti Polar Lipids, Inc., Alabaster, AL.

  Oleoyl (C18:1)-, linoleoyl (C18:2)- and linolenoyl (C18:3)-phosphatidylcholine mixed (400:25:1) and formed into a thin film with cholesterol (CH) and dicetylphosphate (DP) in the ratio of 6:4:1 (PL:CH:DP).

Salt: Sodium chloride.

Buffer: Sodium phosphate, pH 5.6, 20 mM

All other chemicals used in the study were acquired from Sigma–Aldrich Canada Ltd., Oakville, ON.

Methods

In vitro model

Hypotheses:

1. Binders (soy, wheat or deheated mustard) affect oxidation of oxyMb.
2. Binders (soy, wheat or deheated mustard) affect oxidation of oxyMb in the presence of unsaturated polar lipids.

To test hypothesis # 1 oxyMb was mixed with different binders at variuos levels and monitored for oxidation with time. To test hypothesis # 2 liposomes of large, multilamellar vesicles were used. These liposomes were prepared according to Lin and Faustman (1993) and involved encapsulating oxyMb and binders with phospholipids containing unsaturated lipids. Preparation of liposomes was carried out as illustrated in Figure 2. First, the lipids were formed into a thin film on a round bottom flask by drying under vacuum. Then the oxyMb, binder, salt

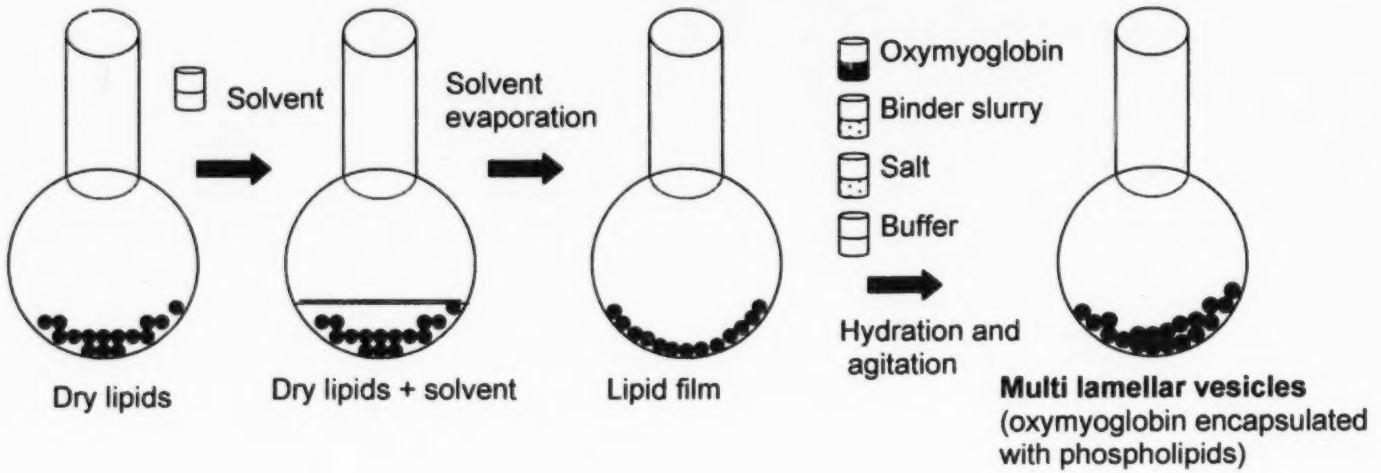

Figure 2. Formation of liposomes for the *in-vitro* model.

and buffers were added and mixed well to encapsulate the aqueous components with lipids and to form a liposome system consisting of multilamellar vesicles. Prepared liposomes were stored at 5°C under normal room light and continuous mixing was ensured. Oxidation of oxyMb and phospholipids in the liposome systems were monitored over time. All experiments were carried out as a minimum of three replications.

Burger-type ground beef product

Hypotheses:

1. Binders (soy, wheat or deheated mustard) influence oxyMb oxidation and metMb formation in ground, mixed beef during refrigerated storage.
2. Binders (soy, wheat or deheated mustard) influence lipid oxidation in ground, mixed beef in refrigerated storage.
3. Binders (soy, wheat or deheated mustard) accelerate red colour change of ground, mixed beef in refrigerated storage.

Table 1. Product formulation

Ingredient	Percentage by weight	
	Control	With binder
Beef *	89.0	89.0
Ice	10.0	5.5
Salt	1.0	1.0
Binder	-	4.5

**Beef inside round, 2% fat, 22% protein, 74% moisture*
Ground using a 11/16" plate first and then a 1/8" plate
Final meat protein content in patties is about 18%

Figure 3 illustrates the flow diagram of forming beef burgers. Ingredients were mixed together and burgers (120 g, 1 cm thick) were formed using the OMAS hamburger press (OMCAN Manufacturing and Distributing Inc., Oakville, ON). Burgers were placed in Styrofoam plates individually and wrapped in an oxygen permeable film (30 000 $cm^3/m^2/24h$,

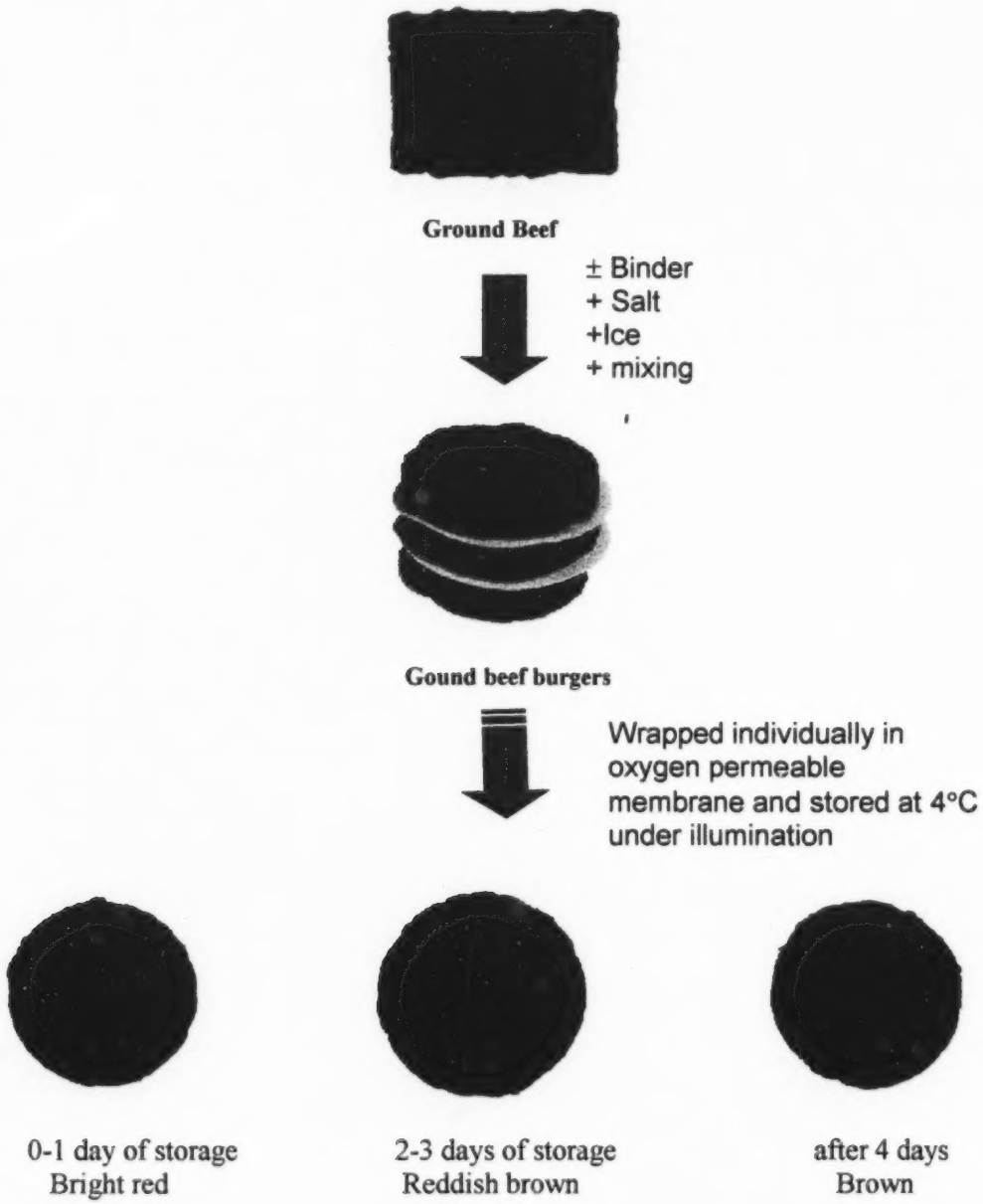

Figure 3. An illustration of fresh beef burger study.

Vitafilm, Huntsman film products of Canada, Toronto, ON) and stored under 990 Lux at 4°C for 5 days. Samples were withdrawn at 24 h intervals. All nine binders were evaluated and a control was prepared without a binder. As an antioxidant, ascorbic acid was evaluated at a 300 ppm level without any binder. Three separate burger trials (for each binder) were used for this study. Data analysis was performed as for a split-plot design using SAS statistical software (Statistical Analytical System, Version 6).

Study on prooxidant and antioxidant activity of binders

Hypotheses:

1. Binders (soy, wheat or deheated mustard) have oxidative enzyme activities that accelerate oxyMb oxidation.
2. Binders (soy, wheat or deheated mustard) have oxidative compounds that accelerate unsaturated lipid oxidation.

In order to test these hypotheses the binders were investigated individually for their oxidative enzyme activities (*i.e.,* catalase, polyphenol oxidase, lipoxygenses), for their radical scavenging abilities (*i.e.,* hydroxyl and lipid peroxy), and for their iron chelating capacity.

Chemical and physical analysis employed

Determination of myoglobin forms in meat and in vitro models

The oxidation of oxyMb and formation of metMb were determined by monitoring the visible spectra of myoglobin obtained from diluted liposomes or meat extracts. Myoglobin was extracted by homogenizing an aliquot of meat in a phosphate buffer (50 mM, pH 6.8 and 1:3,w/v). Insoluble particles were removed by centrifugation (10 000 rpm, 10 min) and the supernatant containing Mb was recovered for quantification. For the *in vitro* models, an aliquot of model solution/liposome was withdrawn at specified time intervals. The visible spectra (400

to 700 nm) of these samples were recorded using a scanning spectrophotometer (HP8350, photodiode array, Agilent Technologies, Wilmington, DE) and forms of Mb were calculated as total, oxy- and metmyoglobin (using absorbencies at 525, 545, 565 and 572 nm) according to the method of Krzywicki (1982).

Determination of extent of lipid oxidation

The buffer extract used for Mb determination or a sample withdrawn from the liposome preparation was utilized to determine 2-thiobarbituric acid (2-TBA) reactive substances (TBARS) as an indicator of lipid oxidation. Proteins of the sample were removed by precipitation with 10% (w/v) trichloroacetic acid followed by centrifugation (10 000 rpm, 5 min). An aliquot of the supernatant was added to the 2-TBA and incubated at 85°C for 30 min to develop the TBARS complex. The extent of chromophore development was determined as absorbance at 532 nm and quantified using a standard curve constructed with 1,1,3,3, tetramethoxypropane. Results are expressed as nmol malondialdehyde equivalents per g of sample (Schmedes and Hølmer, 1989).

Determination of colour values of beef patties

The CIE-L*, a* and b* values of the patties were assessed using the Hunter Lab colorimeter (Miniscan XE, Hunter Associates Laboratory Inc., Reston, VA). Three selected areas in the patties were followed for the L* (light,100; dark, 0), a* (+ red, - green) and b* (+ yellow, - blue) values during the storage period.

Determination of oxidative/antioxidative potential of binders

Antioxidative potential of binders was evaluated as total phenolic content (Yen and Duh, 1994), ability to scavenge hydroxyl radicals (Halliwell *et al.*, 1987) and ability to chelate ferrous ions (Yen and Duh, 1994). Activities of the oxidative enzymes, lipoxygenase, peroxidase and catalase (Garcia *et al.*, 2000), of the binders were also evaluated.

RESULTS AND DISCUSSION

In-vitro model (liposome) study

Binders used in this study showed an influence on oxyMb oxidation even without unsaturated lipids present in the system. All binders that were studied exhibited a protection against the early stage of oxyMb oxidation, but it was lost during storage. Values presented in Table 2 indicate the length of time (h) taken to reduce the initial content of oxyMb by 50%. Toasted wheat crumb (TWC) and micronized whole wheat flour (MWWF) were able to slow down the oxidation as compared to the normal rate, while the other binders produced an acceleration of oxyMb oxidation *in vitro*. Since there were no lipids present in the system, this was an indication of the possible direct effect of binders on influencing the oxidation of oxyMb and acceleration of brown colour formation.

Table 2. Time (h) taken to reduce the initial oxymyoglobin content by 50% in the liposomes.

Binder	Time (h) for 50% reduction of the initial oxymyoglobin concentration	
	Without unsaturated lipids[a]	With unsaturated lipids[b]
No binder (control)	24	8
Micronized whole wheat flour (MWWF)	20	6
Toasted wheat crumb (TWC)	28	6
Laboratory milled wheat flour (UWF)	10	6
Commercial wheat flour(WF)	6	2
Commercial wheat gluten (WG)	10	5
Whole wheat flour (WWF)	20	5
Deheated mustard flour (DMF)	8	3
Soy protein concentrate (SPC)	6	3
Soy protein isolate (SPI)	8	3

[a] *Initial oxyMb content was 0.699 mg/ml*
[b] *Initial oxyMb content was 0.713 mg/ml*

In developing the liposome model, the ratio of saturated, monounsaturated and polyunsaturated fatty acids ratio for the polar lipid fraction of beef intramuscular lipids was

tested. The phospholipids (PL) selected for this study contained oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) which are the predominant unsaturated fatty acids found in polar lipid fraction of muscle tissues. The ratio of these three fatty acids in the polar fraction of beef fat was 20:10:1. When this ratio of PL mixture was employed for the preparation of liposomes, encapsulated oxyMb turned to brown metMb rapidly even during the formation of liposomes. Absence of the endogenous antioxidants may be one of the reasons for faster oxidation of unsaturated phospholipids in the free form and subsequent oxidation of oxyMb in the liposomes. Therefore, different ratios of these three PLs were studied and finally the ratio of 400:25:1 (oleyl:linoleoyl:linolenoyl) was found to give a reasonable working stability for the liposome model.

Liposomes are vesicles and were made by encapsulating water-soluble oxyMb and binder in a thin lipid film containing phospholipids. This membrane system allowed us to examine the oxidative effect of binders on both lipids and oxyMb in an isolated situation. It should be noted here, however, that in a real meat system there are other biochemical changes occurring simultaneously.

According to the liposome study, all binders except toasted wheat crumb accelerated oxyMb oxidation more than that of the control (*i.e.*, with no binders added), but at different rates (Table 2). Among the binders evaluated, soy-based ones and commercial wheat flour accelerated oxyMb oxidation considerably as the time taken for reduction of the initial oxyMb content by 50% was shorter than that of other binders. Among the wheat-based binders, MWWF, UWF and TWC were able to give relatively good protection against oxidation of oxyMb. When lipids were present in the system, all binders resulted in a lower oxyMb content than the control during the period of study. They took a shorter time to reduce the initial oxyMb content by 50% compared to the control without any binder added. Among the binders

evaluated, all wheat-based ones except commercial wheat flour performed better than the soy binders or DMF.

The TBARS values, which are indicators of lipid oxidation, showed that UWF, WG and WF enhanced lipid oxidation (results not shown). When all binders were compared, MWWF, TWC and SPI were found to suppress lipid oxidation in the liposomes. The binders that slowed down lipid oxidation did not, however, necessarily delay oxyMb oxidation in the liposomes (*i.e.*, compared to the model when no binder was added). Hence, it is difficult to accept the supposition that the binders' effect on enhanced oxyMb oxidation is a direct result from accelerated lipid oxidation as this wasn't always observed in the liposome study.

Conclusions:

1. Soy- or wheat-based binders or deheated mustard flour influence oxyMb oxidation even without unsaturated lipids present.
2. Soy- or wheat-based binders or deheated mustard flour influence oxidation of oxyMb when unsaturated lipids are present.
3. Soy- or wheat-based binders influence the oxidation of unsaturated lipids when oxyMb is present.

Fresh beef product study

An examination of the effect of binders on beef offers a more realistic picture on fate of myoglobin *in situ*. The meat used for the product formulation had a very low total fat content (which consisted mostly of intramuscular lipids).

CIE a value*

Beef burgers containing different binders as reflected by CIE a* values showed that, initially the patties lose their redness faster when any of the binders were present. However as the storage continued, WWF, MWWF, UWF and TWC containing burgers were able to maintain a* values close to the control or stayed as higher values than control. Burgers with WG resulted in lower a* values after 24 h of storage, indicating faster red colour deterioration. At day 3 of storage, the control, WG and DMF containing burgers had a* values lower than 18.0; they appeared brown on the surface. Burgers with MWWF and WWF had high a* values even after 4 days of storage. Use of CIE L*, a*and b* values for the evaluation of meat colour is a well-established practice. It has been found that in lean ground beef, CIE a* values less than 16 result when the metMb content is above 40% of the total content (Shivas, 1984). In our study this point was reached when the CIE a* value was around 18.0. Brown metMb becomes apparent when its content is 40-50% of the total heme pigments and consumer rejection occurs when the metMb content is greater than 40% (Shivas et al., 1984). Table 3 provides a summary of the performance of binders in changing redness of beef burgers.

Table 3. Performance of the plant binders in beef burgers according to the CIE a* value[a].

Storage (days)	Performance of binders as CIE a* values ← → *Highest* *Lowest*	Range
0	CONTROL>UWF>MWWF>WWF>TWC>WF>WG>DMF>SPI>SPC	34.8-30.0
1	WWF>MWWF>CONTROL>TWC>DMF>UWF>WF>SPC>SPI>WG	29.8-24.0
2	WWF>MWWF>CONTROL >TWC>UWF>WF>DMF>SPC>SPI>WG	29.8-22.0
3	WWF>CONTROL>MWWF>TWC>SPC>SPI>WF>UWF>**WG>DMF**	26.0-10.8
4	MWWF>WWF>**TWC>SPC>SPI>UWF>WF>CONTROL>WG>DMF**	23.3-9.8

[a]*Binders in bold have CIE a* value less than 18.0. Below this value, the product surface appears considerably brown.*

OxyMb and metMb content

Some of the binders, notably micronized- and whole wheat-flours, at a 4.5% (w/w) level of incorporation were able to slow down the rate of oxyMb oxidation compared to that of the control without any binder added. Table 4 gives the changing pattern of oxyMb content of beef burgers in relation to burgers with added binders. The total Mb content of the burgers varied from 0.91 to 0.94 mg/ml of extract. It is interesting to note here that DMF containing burgers resulted in a higher oxyMb content than other binder-containing burgers during day 0 and 1 of the study. The oxymyoglobin content and CIE a* value of the meat have a positive relationship. Those binders which resulted in high a* values had a high oxyMb content. Here also it is noted that burgers with WG added were unable to retain oxyMb levels compared to burgers with the other wheat-based binders.

Figure 4 illustrates the relationship between metMb content and a* value of the burgers containing different binders and stored for a 5-day period. It is interesting to note the distribution of each binder and differences among storage days. The dotted lines indicate an a* value of 18 and metMb content of 50%. The samples that are enclosed in the circle had higher a* values and also lower metMb content than the rest. All the day 0 samples (with or without binders), day 1 samples (all others except for WG) and WWF- and MWWF-containing burgers that were stored for 3 days are in this group. All data indicate that MWWF and WWF contribute to a retardation in metMb formation or oxyMb oxidation in ground beef products.

Lipid oxidation

Although it was expected that lipid oxidation of beef patties may follow a similar pattern as that of oxyMb oxidation, this was not so. Burgers containing soy-based ingredients and heat-

Table 4. Performance of plant binders in beef burgers according to oxyMb content (mg/ml)[a].

Storage time, days	Performance of binders as oxymyoglobin content[b] ← Highest … Lowest →	Range
0	DMF>AA>TWC>CONTROL>MWWF>WWF>WF>WG>SPI>UWF>SPC	0.87-0.72
1	DMF>SPC>WWF>TWC>AA>WF>MWWF>CONTROL>UWF>SPI>WG	0.77-0.40
3	MWWF>WWF>TWC>AA>WF>UWF>CONTROL>SPC>WG>DMF>SPI	0.46-0.22
4	AA>MWWF>UWF>WWF>WF>CONTROL>WG>TWC>SPC>SPI>DMF	0.41-0.13

[a]*High oxyMb content-indicates red colour.*
[b]*AA - ascorbic acid*

treated mustard flour exhibited a very low extent of lipid oxidation compared to other combinations tested. These binders did not however delay oxyMb oxidation compared to some other binders that actually slowed down brown colour formation. MWWF resulted in moderate suppression of lipid oxidation products. Table 5 shows how the binders performed according to their ability to suppress lipid oxidation in fresh beef burgers.

Table 5. Performance of plant binders according to the TBARS values[a].

Storage time, days	Performance of binders as TBARS, nmol MDA equivalents[b] ← Lowest … Highest →	Range
0	DMF>WWF>SPC>SPI>TWC>AA>MWWF>WG>CONTROL>WF>UWF	2.4-5.9
1	DMF>SPC>WWF>SPC>MWWF>AA>TWC>WF>CONTROL>WG>UWF	1.9-13.7
3	DMF>AA>WWF>SPC>SPI>MWWF>TWC>CONTROL>WF>WG>UWF	3.1-17.0
4	DMF>AA>SPI>WWF>SPC>MWWF>TWC>CONTROL>WF>WG>UWF	1.1-24.6

[a]*Low TBARS value indicates low lipid oxidation.*
[b]*AA - ascorbic acid*

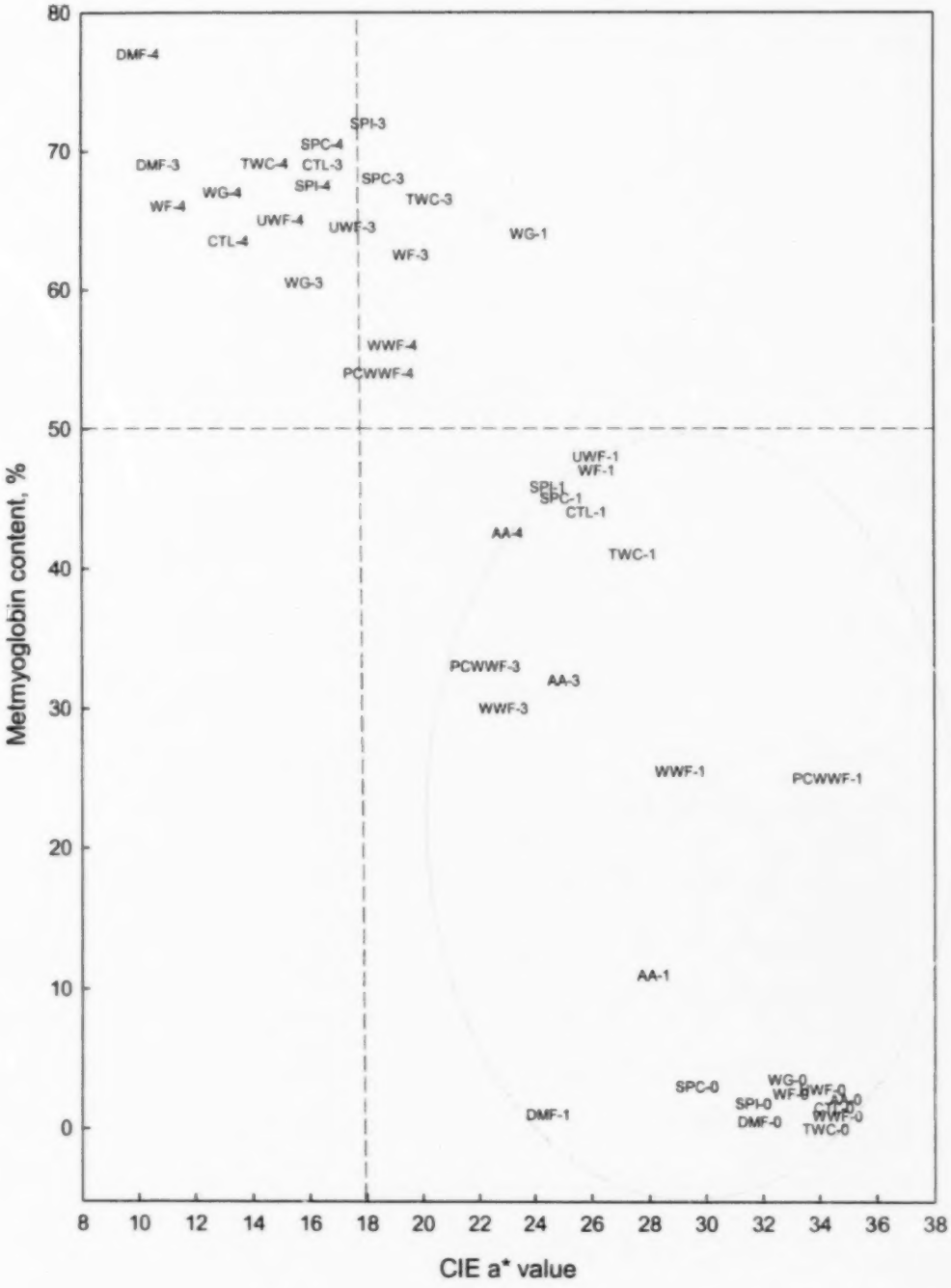

Figure 4. Relationship of metmyoglobin content and CIE a* value of the beef burgers. Samples inside the circle have lower metmyoglobin content and high a* values and are not brown in colour.

In addition to the binders, incorporation of oxygen scavenging antioxidants (water soluble ascorbic acid and Trolox, and lipid soluble alpha-tocopherol) to beef was studied to see the effect on colour and conversion of Mb. Ascorbic acid at 300 ppm was able to slow down the rate of oxyMb oxidation considerably compared to the other two antioxidants and the control. Visual colour evaluation also proved that the beef burgers retained their red colour until day 6 of storage while the control, and burgers containing 300 ppm tocopherol and Trolox started to turn to brown on day 2. Ascorbic acid resulted in very low lipid oxidation products by giving minimum TBARS value throughout the storage study. Ascorbic acid is an additive that is used as a cure accelerator in meat (used under good manufacturing practice) but is not commonly used in fresh meat products. However, it shows that the combination effect of reducing activity and reactive oxygen scavenging ability of ascorbic acid is very effective in maintaining fresh red colour of beef burgers.

Addition of these binders did not markedly alter the pH of meat during the storage period Starting pH of the meat was around 5.5 and it was reduced to 5.3 and 5.4 by most of the binders except DMF and TWC. These two binders caused a slight increase in pH of meat.

The results of this study indicate that there are certain components in some of the binders (*e.g.*, WG) that accelerate both lipid and oxyMb oxidation while some accelerate only one type of oxidation (*e.g.*, DMF, SPI and SPC suppress lipid oxidation but accelerate red colour degradation). Evaluation of the antioxidant/proxidant potential of the water-soluble fraction of binders gave an indication of their behaviour as summarized in Figure 6. The binders that had higher values for total phenolic content (*i.e.*, compounds that can act as antioxidants) and high levels of hydroxyl radical scavenging actually inhibited lipid oxidation; examples are DMF, SPC and SPI. These are not good inhibitors of oxyMb oxidation. Presence of these components at

high levels may negatively affect oxyMb oxidation. The wheat ingredients were moderate in these activities.

Conclusions:

1. Soy binders and deheated mustard flour accelerate red colour change of ground, mixed beef during refrigerated storage. Among the wheat-based ingredients, micronized wheat flour and toasted wheat crumb can maintain the redness of the product better than other binders or control.
2. Soy- or wheat-based binders influence oxyMb oxidation and metMb formation in ground, mixed beef during refrigerated storage. Among these, micronized, whole-wheat flour and toasted wheat crumb may slowdown the metMb formation.
3. Soy- or wheat-based binders influence lipid oxidation in ground, mixed beef during refrigerated storage. Soy protein concentrate and isolate and deheated mustard flour are very effective in reducing oxidation of unsaturated lipids of fresh beef.

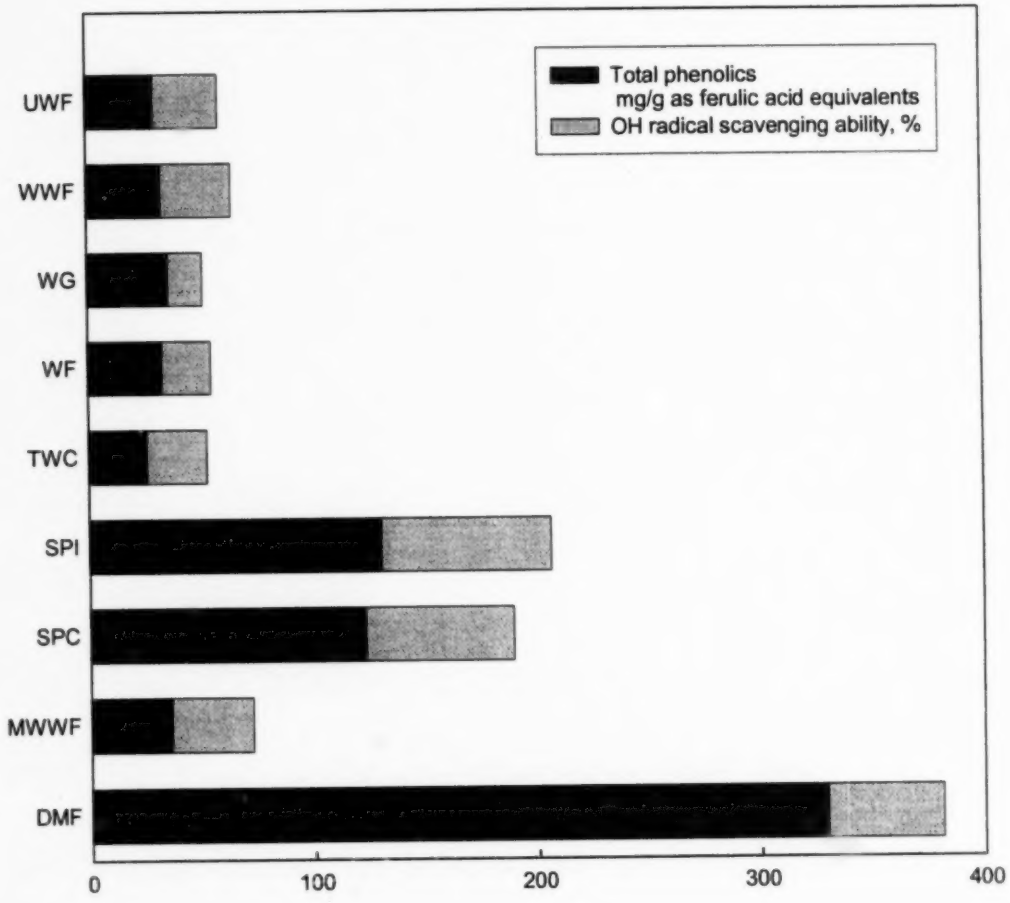

Figure 5. Total phenolic content and hydroxyl radical scavenging activity of binders. A 4.5 % (w/w) slurry of the binders was used for these determinations.

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PERSONNEL PAID BY THE GRANT:

L.L. Kok – 9 months (April 2001 to December 2001)
P.K.J.P.D. Wanasundara – 14 months (January 2001 to February 2002)

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Manuscripts in preparation:

Title - Oxidation of oxymyoglobin and unsaturated lipids of comminuted meat mixed with plant binders.
Proposed Journal – Food Chemistry

Title - Do plant-based binders affect colour of fresh comminuted meat products?
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